

Acidic Microbe Community Fosters the Unique

Mount Shasta, California

WEST of Mount Shasta in northern California, some of the most acidic water found on Earth lies deep underground. Rich in dissolved metals, the water is leaching from the old Richmond Mine at Iron Mountain. The pH of the mine's water has been found as low as -3.6 in some places, far more corrosive than battery acid.

An environment so inhospitable to humans makes quite a cozy home for certain microbes, called extremophiles for their ability to survive in harsh conditions. Studying extremophiles in the laboratory has been challenging because of the difficulty of re-creating their natural environments. In fact, most of what is known about microbes has come from just a few that can be successfully cultured in the laboratory, such as *Escherichia coli* and yeast. Microbial systems account for a huge percentage of Earth's biomass and play key roles in Earth's biogeochemical cycles, yet they are poorly understood.

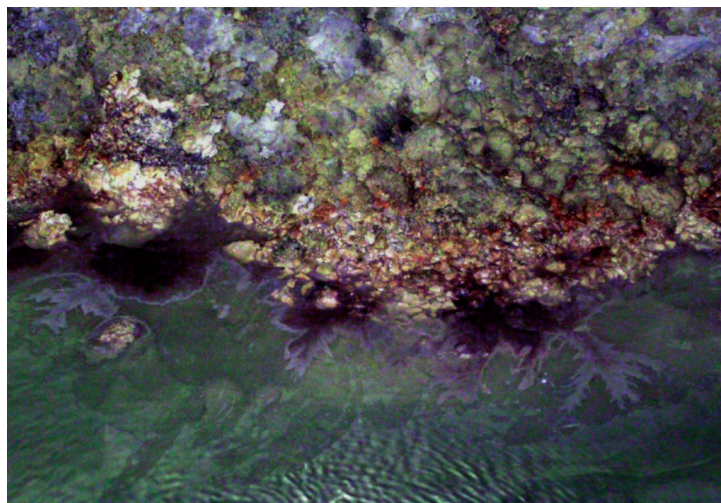
The genomic revolution, beginning with the completion of the Human Genome Project, has changed the study of microbes entirely. In the last two years, researchers have learned how to tease genetic information directly from an environmental sample of microbes. As it turns out, subsurface acid mine drainage ecosystems are excellent models for genomic studies of microbial ecology and evolution. These acidophilic (acid-loving) communities are self-sufficient, physically isolated, and relatively simple both geochemically and biologically.

"Now that we can examine whole communities—not just cultured samples—we are finding far more diversity among microbes than anyone had suspected," says Livermore biochemist Michael Thelen. He is leading a team of Livermore scientists and collaborating with researchers from the University of California (UC) at Berkeley and Oak Ridge National Laboratory to take microbial studies beyond sequencing and gene analysis. Their examination of microbial communities in the Richmond Mine has revealed hundreds of unique and unusual proteins.

Down in the Mine

At a junction in the Richmond Mine referred to as the "5-way," an access tunnel intersects four tunnels within the ore deposit, about 1 kilometer underground. Virtually all drainage from the mine moves through the 5-way, where the pink film on the water's surface teems with acidophilic microbes. The pH of the water at the 5-way is about 1, and the water's temperature is about 42°C (107°F).

Jillian Banfield of UC Berkeley has been studying the Iron Mountain site and its biofilms for 10 years. In 2003, a Richmond Mine biofilm from the 5-way was analyzed at the Joint Genome Institute (JGI) in Walnut Creek, California, in the first-ever sequencing of DNA from an environmental microbial



In Iron Mountain, pyrite (also known as fool's gold) interacts with microbes, oxygen, and water to create hot sulfuric acid laden with heavy metals.

community. Sequencing revealed a relatively simple consortium of three bacteria and three archaea (microorganisms of harsh, hot acidic environments).

Each microbial community in the Richmond Mine contains a consistent set of organisms but with varying numbers and strains of bacteria and archaea. “Each strain has adapted to the particular conditions of its site,” notes Thelen. But the causes for the differences—such as a dependence on a certain mineral, competition, or cooperation—are not yet known.

In 2004, Thelen spent a sabbatical year at UC Berkeley working with Banfield. Since then, he and his collaborators have analyzed hundreds of proteins in Richmond Mine biofilms using computational biology, mass spectrometry-based proteomics, and biochemical methods. They are not only identifying novel proteins but are also defining the essential functions of each protein. Some proteins are important for signaling, defense, or transport. Others, known as cytochromes, transfer the electrons gained from iron oxidation. At Iron Mountain, the team has discovered several cytochromes containing novel heme groups that specifically form a coordination complex with iron.

Proteins at Work

A sequence database of over 12,000 proteins was created from the biofilm genomic data set. From this database, the Oak Ridge collaborators identified protein spectra in biofilm extracts using nanoliquid chromatography tandem mass spectrometry. In most cases, proteins could be tracked to specific organisms, because the genes that encode them are on DNA fragments that have already been assigned to organism types. More than 2,000 proteins were determined to be from the five most abundant biofilm organisms.

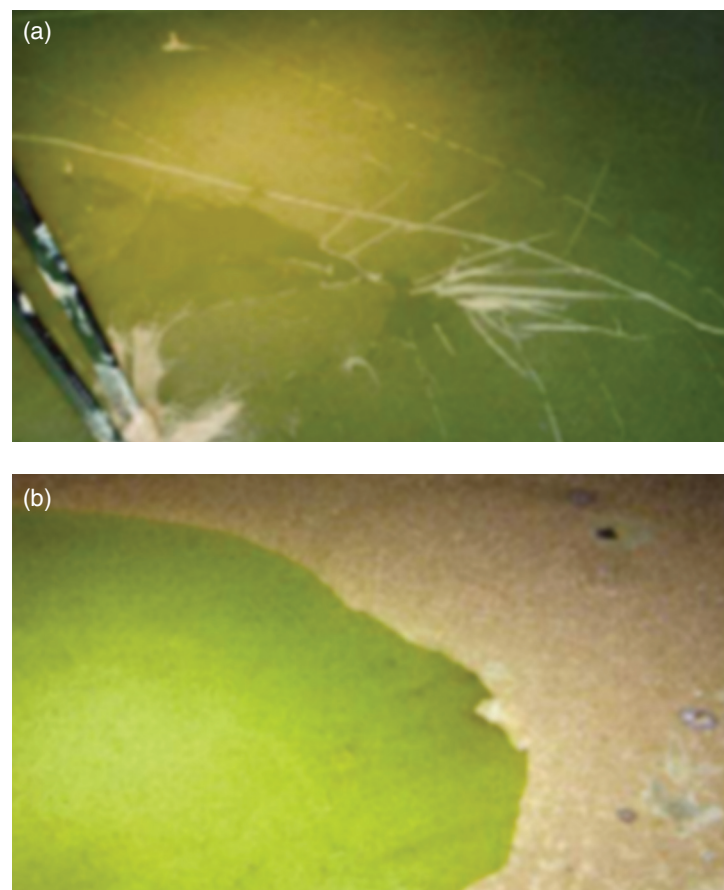
Analysis of abundant known proteins revealed the importance of protein refolding, perhaps to achieve stability in a highly acidic environment. Other abundant proteins defend against oxidative stress, an important challenge in the acid mine drainage environment.

Overall, however, the biofilm was dominated by proteins that are products of genes originally annotated as “hypothetical” when the genomic data set was established (42 percent of the genes in the original data set). These genes have no known function, and their protein products have not been confirmed in any way. Among the abundant hypothetical, or novel, proteins, 15 percent were unique (not significantly similar to any known protein) and

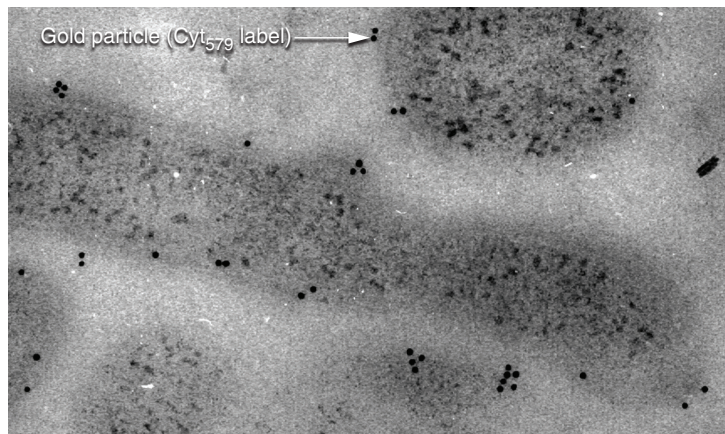
2 percent were conserved (similar to other predicted but not yet characterized proteins).

In the extracellular portion of the biofilm, 52 percent of proteins were unique and about 14 percent were conserved novel proteins. These proteins may be important for adaptation to the extremely acidic and metal-rich conditions.

Two of the new extracellular proteins were identified as cytochromes, which contain the heme-iron complex. The spectra



In January 2004, samples of biofilm were collected from the Richmond Mine at Iron Mountain, near Mount Shasta in California. (a) The biofilm occurs as a continuous sheet over the surface of the acid mine drainage pool; wrinkles form because of movement in the solution. (b) In this close-up photograph taken during sample collection, the biofilm is thick and appears to be homogeneous.

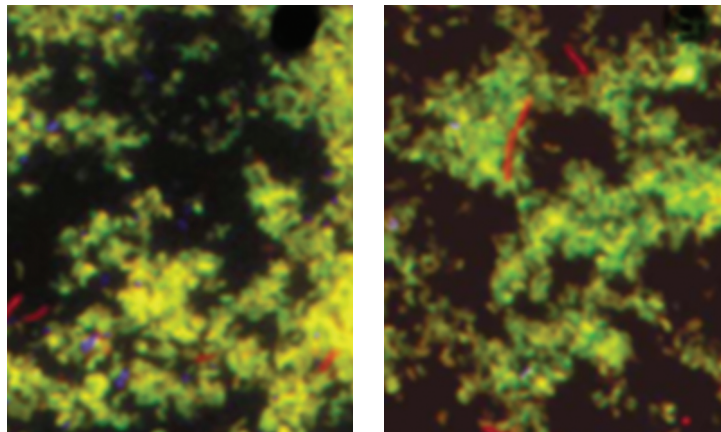


Cyt₅₇₉ is a protein actively involved in iron oxidation. The protein's location outside the *Leptospirillum* bacteria is shown here, where an antibody labeled with gold binds to its target cytochrome protein.

of these two proteins contain peaks at 572 and 579 nanometers—unique among the hundreds of known cytochromes—so they have been dubbed Cyt₅₇₂ and Cyt₅₇₉. Thelen's laboratory analyses showed that both have unusual heme characteristics and are actively involved in iron oxidation. Because *Leptospirillum* group II dominates most biofilms from the Richmond Mine and is frequently detected at other mining sites, the team predicts that these cytochromes are two of the key enzymes connecting the biology and geochemistry of metal-rich acidic environments.

The team was able to assign a probable function to 69 percent of the detected *Leptospirillum* group II proteins on the basis of sequence similarity. It turns out that, as in so many communities, individuals or groups of individuals take responsibility for various activities on behalf of the entire group. In the Richmond Mine biofilms, nitrogen fixation for all members of a community is handled by proteins in *Leptospirillum* group III, the less abundant bacterium. Similarly, carbohydrate metabolism is considerably greater in *Leptospirillum* group III than in *Leptospirillum* group II.

Whether this division of labor has always been this way or has evolved over time is unknown. "We're interested in how these species have adapted to the extreme geochemical conditions in the iron mine ecosystem," says Thelen. "We want to know whether these microbes have been selected as a community by the environment as well as how they evolve with the changes caused by seasonal and other geochemical fluctuations."



Fluorescence in situ hybridization analyses of the collected Iron Mountain biofilm show the *Leptospirillum* protein group II (yellow) and other bacteria (red).

Other Extremophiles, Too

Team members are now working with Everett Schock, a geochemist from Arizona State University, to examine mats of thermophilic microbes in the boiling hot springs of Yellowstone National Park. They hope to isolate, identify, and begin to understand the proteins of microbes that interact with silicate, the most abundant mineral in the hot springs environment.

A long-term goal of Thelen's work is to apply new information about microbial decomposition processes to the creation of new sources of energy from plant matter. The hope is that microbes could someday speed the transformation of the lignin in biomass to sugar and then to usable ethanol.

Such studies are part of the Department of Energy's Genomes to Life Program, an effort to better understand the mechanisms of life, beginning with microbes. Microbes are already being used to clean up contaminated soil, and their usefulness for energy production, carbon sequestration, and cleaner industrial processes may eventually be established. Better living through microbes.

—Katie Walter

Key Words: acid mine drainage, archaea, bacteria, genomics, iron oxidation, *Leptospirillum*, proteomics.

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